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Molecular Characterization of Camberwell Virus and Sequence Variation in ORF3 of Small Round-Structured (Norwalk-Like) Viruses

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Five small round-structured viruses (SRSVs) associated with gastroenteritis in Victoria, Australia, from January to November 1994 were examined by sequencing cDNA prepared from faecal samples using RT-PCR. The sequence of the 3′ half (3.8 kb) of the genome of one of these viruses, Camberwell, was determined. Camberwell virus was related most closely to Bristol and Lordsdale viruses, and belonged to the genetic group of SRSVs containing Bristol, Lordsdale, Toronto, OTH-25, Mexico, and Hawaii viruses. The amino acid identities between Camberwell and Bristol viruses for proteins encoded by ORF1 (partial), ORF2, and ORF3 were 99%, 98%, and 90%, respectively. A highly variable region in ORF3 corresponding to amino acid residues 123 to 169 (Bristol and Camberwell numbering) were identified. Short segments of ORF1 (polymerase region) and the highly variable ORF3 region was analysed for the other four viruses. The results obtained indicated the potential usefulness of the variable region in distinguishing between closely related viruses.

KEY WORDS: SRSV, human enteric calicivirus, sequence variation

INTRODUCTION

Norwalk and related viruses belong to the group of small round-structured viruses (SRSVs) that are associated with epidemics of viral gastroenteritis in adults and older children. The Norwalk-like viruses detected in faeces are spherical in shape, 27–40 nm in diameter, and lack sharply defined edges [Greenberg and Matsui, 1992]. The capsid contains a single polypeptide of 59,000 M_r [Greenberg et al., 1981]. Studies using human convalescent sera established the existence of several serotypes, but uniform nomenclature is not used at present because of the lack of standardized tests and a suitable range of immunological reagents [Lewis, 1991; Lewis et al., 1995; Lambden et al., 1993; Wang et al., 1994]. Virus replication in cell culture or disease in an animal model has not been demonstrated.

The sequencing of the single-stranded RNA genome of three viruses, namely, Norwalk [Jiang et al., 1993], Southampton [Lambden et al., 1993], and most recently Lordsdale [Dingle et al., 1995], is completed and facilitated the classification of the viruses into the family *Caliciviridae* [Cubitt et al., 1994]. The genome is of positive polarity and 7.5 to 7.7 kb in length. There are three major open reading frames (ORFs). ORF1 is the longest, encoding approximately 1,700 amino acids. Comparisons with other positive-strand viruses led to the identification of sequence motifs corresponding to a helicase and an RNA polymerase. ORF2 encodes the capsid protein and partially overlaps ORF1. The product of ORF3 has not been identified and its function is unknown.

Partial sequences ranging in length from 150 to 3,000 nucleotides and located in the 3′ half of the genome have recently been published for a number of Norwalk-like viruses. The viruses were obtained originally in widely differing geographic locations from disease outbreaks occurring many years apart over the period since 1971 [Norcott et al., 1994; Green et al., 1994; Lew et al., 1994a–c; Wang et al., 1994; Green et al., 1994; Utagawa et al., 1994; Jiang et al., 1995]. Several conclusions arise from the analysis of these partial sequences. First, the locations of ORF1, ORF2, and ORF3 fit the pattern of Norwalk and Southampton viruses. Second, there is considerable diversity in the deduced amino acid sequences for ORF2 and ORF3. Third, viruses may be divided into at least two groups based on limited ORF1 (polymerase) sequencing [Ando et al., 1994; Green et al., 1994; Lew et al., 1994b; Norcott et al., 1994; Carter and Cubitt, 1995]. To detect additional genetic groups and to determine the variation within the known ones, the sequences of more isolates are required. The information will enable the correlation of serotypes and genotypes, and con-

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tribute to understanding the epidemiology and evolution of the viruses. Such an approach has been used successfully in the study of RNA viruses belonging to other families. In this paper we present and analyze the sequences of ORF2, ORF3, and part of ORF1 for Camberwell virus, a SRSV closely related to Bristol and Lordsdale viruses [Green et al., 1994; Dingle et al., 1995]. Bristol and Lordsdale viruses were associated with outbreaks of gastroenteritis in 1993 (in UK); Camberwell virus was associated with an outbreak in early 1994 (in Australia). We also document genetic variation in a selected segment of ORF3 for four additional virus isolates associated with outbreaks of vomiting and diarrhoea in Victoria, Australia. These viruses were closely related to Camberwell virus and were detected during an 11 month period.

MATERIALS AND METHODS

Source of Faecal Samples

Faecal samples for this study were derived from five separate gastroenteritis outbreaks termed Camberwell, Rowsley, Upper Yarra, Frankston, and Gisborne after the areas in which they occurred. These locations are all within a radius of 60 km from central Melbourne in the state of Victoria. The main features of these outbreaks were as follows.

Camberwell. In January 1994 an outbreak of vomiting and diarrhoea was reported in a hostel for the aged in Camberwell. A total of 30 residents and 8 staff were affected by the illness. The duration of the illness was 24–36 hr with the major symptoms of vomiting and diarrhoea occurring during the first 6 hr. The outbreak lasted for 10 days. The virus isolate is designated Camberwell-101922/94/AUS following the suggested convention of Wang et al. [1994].

Rowsley. An outbreak of gastroenteritis occurred in a recreational camp in March and April 1994. The main symptoms were vomiting and nausea although some individuals had diarrhoea. The virus isolate is Rowsley-118717/94/AUS.

Upper Yarra. Thirteen out of 27 school children complained of vomiting and diarrhoea in July 1994 at a school camp in the Upper Yarra. Virus is Upper Yarra-138513/94/AUS.

Frankston. In September 1994, 57 out of 87 residents and 12 staff members of a hostel for the aged reported vomiting and/or diarrhoea. Subsequently the illness spread to a hospital where one of the patients from the initial outbreak had been transferred for rehydration. In the second outbreak 17 of 24 patients and 10 staff were affected. The incubation period for both outbreaks was about 24 to 48 hr, with the illness lasting between 24 and 72 hr. Virus is Frankston-138534/94/AUS.

Gisborne. An outbreak of gastroenteritis took place in an aged-care hostel in November 1994. Thirteen of the 30 patients in the hostel and 5 of the 7 staff became ill. The illness was characterised by vomiting, headache, and diarrhoea. Patients generally became well in 24 to 36 hr. Virus is Gisborne-169506/94/AUS.

Faecal samples from these outbreaks were forwarded to Fairfield Hospital for testing for viruses by electron microscopy.

Preparation of Faecal Samples

Faecal samples were purified and concentrated following the method of Oliver et al. [1985]. Briefly, a 20% (w/v) faecal suspension was clarified by two low-speed centrifugation spins. Virus in the clarified fluid was then pelleted through a 45% (w/v) sucrose cushion by centrifugation at 150,000*g* and resuspended in 2 mM Tris-HCl, pH 7.

Electron Microscopy

Purified concentrated faecal samples were stained negatively with 3% phosphotungstic acid (pH 7) using 400-mesh Formvar-carbon coated grids and examined in a Philips CM12 STEM electron microscope. Virus particles were photographed and measured from photographic negatives. Catalase crystals, with half the principal lattice spacing taken to be 8.6 nm, were used as calibration standards. Samples containing SRSV with the characteristic appearance and diameter of Norwalk-like particles were used for RT-PCR. The SRSVs appeared as round, granular staining particles commonly with a fringe of short spikes (Fig. 1). Particles from the individuals tested by RT-PCR from the Camberwell, Rowsley, Upper Yarra, Frankston, and Gisborne outbreaks measured, respectively (mean ± standard deviation): 34.6 ± 0.5 nm (n = 11), 36.5 nm (n = 1), 34.4 ± 1.6 nm (n = 10), 33.8 ± 1.2 nm (n = 9) and 35.2 ± 1.8 nm (n = 5) in diameter.

Reverse Transcription and PCR (RT-PCR)

Viral samples previously concentrated by centrifugation were heated at 99°C for 5 min to release and denature RNA. The procedures for reverse transcription and PCR were essentially those described by De Leon et al. [1992]. AMV reverse transcriptase and *Taq* DNA polymerase were purchased from Promega Corporation and Boehringer Mannheim Australia Pty. Ltd., respectively. DNA was amplified using 35 cycles of 94°C, 50°C, and 72°C (1 min at each temperature). The final extension step was for 7 min at 72°C.

Primers

Random hexameric primers were used for reverse transcription. To obtain cDNA for Camberwell virus, the primers used initially for PCR were 3 and 51 [Moe et al., 1994]. These primers hybridize to the region of ORF1 encoding the putative polymerase and amplify DNA of 206 bp (nucleotides 4673 to 4878 in the Norwalk sequence). Subsequently, fragments of 500 to 600 bp were amplified using the approach of Struck and Collins [1994] to extend in the 3′ and 5′ directions. Primers were designed using the newly determined sequence for Camberwell virus and the published sequences of Southampton, Norwalk and Toronto viruses [Lambden et al., 1993; Jiang et al., 1993; Lew et al., 1994a]. Adjoining fragments overlapped by 50–150 bp.

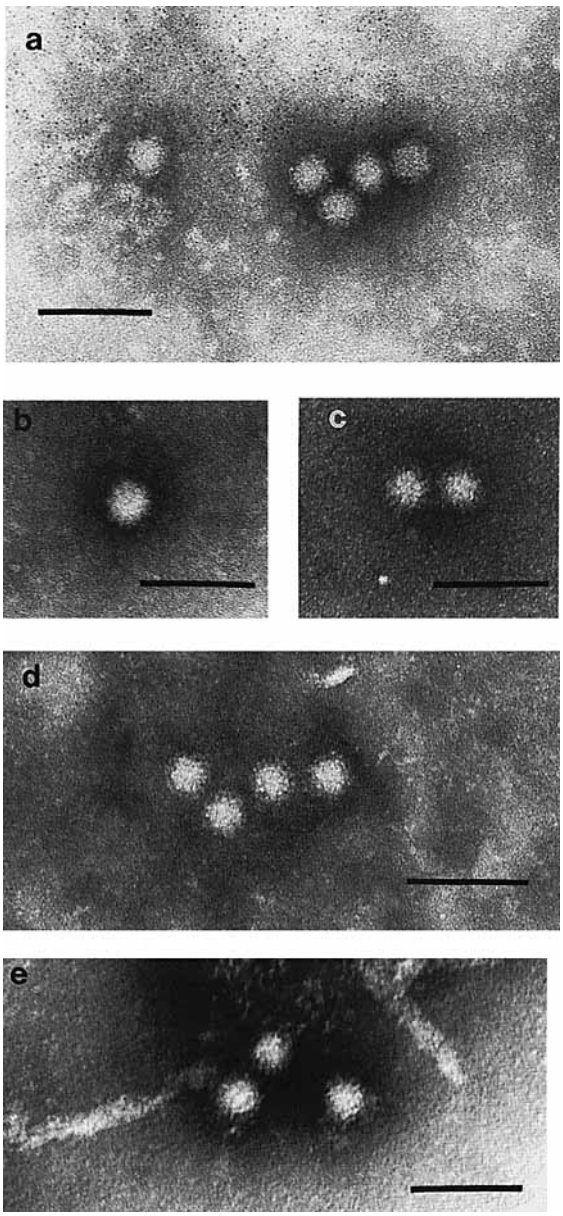

Fig. 1. Negative staining electron micrographs of Norwalk-like particles in the faeces of individuals from the five gastroenteritis outbreaks analysed in this study: **a)** Camberwell, **b)** Rowsley, **c)** Upper Yarra, **d)** Frankston, and **e)** Gisborne viruses. Bars represent 100 nm.

To amplify short segments of Rowsley, Gisborne, Upper Yarra and Frankston viruses, the following primers were used: for ORF1, primers 51 (see above) and 2493 (modified primer 3: 5′-CACCAagTGAG(g/A)T(t/G)-GATGT-3′, changes shown in lower case, degeneracies in parentheses): for ORF3, primers 2720 (5′-ATGA-CAAAGGCTCTGGACTGGAG-3′) and 2721 (5′-AGA-ACCAGCTGAAGAACCTAGTCT-3′). The latter pair amplified cDNA (240 bp) corresponding to ORF3 nucleotides 313 to 552 of Bristol and Camberwell viruses.

DNA Sequencing and Sequence Analysis

The DNA amplified by RT-PCR was purified by electrophoresis through 2–2.5% agarose gels. It was then sequenced without cloning using the Applied Biosystems PRISM™ DyeDeoxy™ Terminator Cycle Sequencing Kit and Model 373A Automated Sequencer. Approximately 95% of the DNA was sequenced on both strands. In the few regions at the ends of amplified fragments where the second strand was not completed, the sequence was clear and unambiguous.

Nucleotide and deduced amino acid sequences were analysed with GeneJockeyII software (Biosoft, Cambridge, UK), which uses the Clustal algorithm of Higgins and Sharp in generating multiple alignments of related sequences.

The SRSVs that were compared with Camberwell virus, and their corresponding GenBank accession numbers were as follows: Bristol B493 (X76716) [Green et al., 1994], Toronto MV24 (U02030) [Lew et al., 1994a], OTH-25/89/J (L238300) [Wang et al., 1994], Hawaii (U07611) [Lew et al., 1994c], Norwalk (M87661) [Jiang et al., 1993], Southampton (L07418) [Lambden et al., 1993], Desert Shield DSV395 (U04469) [Lew et al., 1994], KY-89/89/J (L23828) [Wang et al., 1994], Chiba (D38547) [Utagawa et al., 1994], Mexico (HCU22498) [Jiang et al., 1995], and Lordsdale (X86557) [Dingle et al., 1995] viruses.

RESULTS AND DISCUSSION

The sequence of 3,868 nucleotides of the Camberwell virus genome was determined, corresponding to nucleotides 3923 to 7595 of the Norwalk virus genome. This region contains the 3′ terminal 1,443 nucleotides of ORF1, and all of ORF2 and ORF3. ORF1 and ORF2 overlap by 20 nucleotides as for Bristol and Lordsdale viruses [Green et al., 1994; Dingle et al., 1995]; ORF2 and ORF3 overlap by one nucleotide as for all other sequenced Norwalk-like viruses. The sequence has been submitted to GenBank and assigned the accession number U46500.

The nucleotide and deduced amino acid sequences of ORF1 were compared with the published sequences of ten viruses for which substantial ORF1 sequence information was available. A comparison of the deduced amino acid sequences is shown in Table I. It was clear that Camberwell virus was most closely related to Bristol and Lordsdale viruses, and belonged to a genetic group containing Mexico, Toronto, OTH-25, and Hawaii viruses. Snow Mountain virus also belongs to this group [Wang et al., 1994]. There were only six and seven differences in the deduced amino acid sequences between Camberwell and Bristol, and between Camberwell and Lordsdale viruses respectively, and these were generally conservative in nature. None of the changes were located in conserved RNA polymerase motifs [Green et al., 1994]. A second genetic group contained Norwalk, Southampton, Desert Shield and KY-89 viruses (Table I).

The analysis of the complete ORF2 (capsid) gave results similar to those obtained for the partial ORF1. The

TABLE I. Percentage Identities in Pairwise Comparisons of Partial Amino Acid Sequences Encoded by ORF1 of SRSVs*

	Cam	Bri	Lor	Mex	Tor	OTH	Haw	Nor	Sou	Des
Camberwell 480 aa										
Bristol 475 aa	*99*									
Lordsdale 475 aa	*98*	*100*								
Mexico 294 aa	*88*	*87*	*87*							
Toronto 289 aa	*92*	*91*	*91*	*97*						
OTH-25 (Japan) 289 aa	*91*	*90*	*90*	*96*	*98*					
Hawaii 267 aa	*97*	*96*	*96*	*88*	*91*	*89*				
Norwalk 480 aa	64	64	64	59	62	62	64			
Southampton 480 aa	64	64	64	60	64	63	64	*91*		
Desert Shield 271 aa	63	63	63	59	62	62	63	*83*	*84*	
KY-89 (Japan) 288 aa	63	63	63	60	63	63	64	*99*	*93*	*83*

*The lengths of sequences compared are indicated by the number of encoded amino acids in column 1. All sequences terminated at the carboxyl end of the deduced amino acid sequence. The percentages (adjusted to the nearest whole number) indicative of the genotypic groupings are shown in italics.

TABLE II. Percentage Identities in Pairwise Comparisons of Amino Acid Sequences Encoded by the Complete ORF2 of SRSVs*

	Cam	Bri	Lor	Mex	Tor	OTH	Haw	Nor	Sou	Des
Camberwell 539 aa										
Bristol 539 aa	*98*									
Lordsdale 539 aa	*98*	*100*								
Mexico 548 aa	*64*	*64*	*64*							
Toronto 548 aa	*65*	*64*	*65*	*97*						
OTH-25 (Japan) 548 aa	*64*	*64*	*64*	*96*	*97*					
Hawaii 535 aa	*63*	*63*	*63*	*71*	*71*	*70*				
Norwalk 530 aa	38	38	38	40	41	40	41			
Southampton 546 aa	36	37	37	34	35	35	37	*65*		
Desert Shield 544 aa	38	37	37	38	39	38	40	*63*	*64*	
KY-89 (Japan) 530 aa	37	37	37	39	40	39	40	*96*	*64*	*62*

*The complete length of ORF2 of each virus is indicated by the number of encoded amino acids in column 1. The percentages (adjusted to the nearest whole number) indicative of the genotypic groupings are shown in italics.

viruses examined could be divided into two groups, and Camberwell, Bristol, and Lordsdale viruses were closely related (Table II). Most of the ten amino acid differences between the Australian and these two UK viruses were once again conservative, and scattered throughout the sequence (not shown). Only three were located in the central variable region of the capsid protein identified in comparisons among other caliciviruses. This region corresponds to amino acids 277 to 416 of Camberwell virus and to amino acids 281 to 406 of Norwalk virus.

Fewer complete sequences are available for ORF3 than for ORF2. Table III contains the comparisons of Camberwell ORF3 with six other complete ORF3s and three partial sequences. There are changes from the

```
     1
Cam  MAGAFFAGLASDVLGSGLGSLINAGAGAINQKVEFENNRKLQQASFQFSSNLQQASFQHD
Bri  ..................................................T.........
Lor  ..................................................T.........
Mex  .....I....G.M.TNTV...V....N......D....KY.RN...N-----------..
Tor  .....I....G.M.TNTV...V....N......D....KY..N...N-----------..
Haw  .....I....G.IVTNSV...V....N......D....KQ......N-----------..
Nor  ..Q.IIGAI.ASTA..A..AG.QV.GE.A-----------..SQRY.QNLQ..EN..K..
Sou  ..Q.IIGAI.ASAA..A..AG.Q...E.A-----------..SQRY.QDLA..RNT.E..
Des  ..Q.I.GAI.ATAA..PS.AG.Q..TE.A-----------..HQR..QDLT..SN..K..
Chi  ..Q.IIGAI.ASAA..A..AG.Q...E.A-----------..AQRY.QDLT...N..N..

     61                                                       ->
Cam  KEMLQAQIEATQKLQQGLMKVKQAMLLEGGFSTTDAARGAINAPMTKALDWSGTRYWAPD
Bri  ...............D.......V........A..........................
Lor  ...............D.......V........A..........................
Mex  ....N...A..KR..ADMIAI..PV.TLAA.PLL-MQHV........V...........N
Tor  ....N......KR..ADIIAI..GV.TLAA.PLL-MQHV........V...........N
Haw  ........Q..KQ..ADIIALR.GV.T.
Nor  R..IGY.V..SNQ.LAKNLATRYSL.RA..LTSA....SVAG..V.RIV..N.V.VS..E
Sou  .D..SY.VQ.SNA.LAKNLNTRYS..IA..L.SA..S.AVAG..V.RLI..N.T.VA..R
Des  ...IGL.VG.STA.L.NSLNTRYNL.TQA.M.......AVVG..T.RVV..N.T.IA..M
Chi  ....GY.M.MSN..LAKNLNTRYSL.QA..L.SS....AVAG..V.RLV..G.V.VA..Q

     121                                                      <-
Cam  ARTTTYNAGRFSTSQPSGALPGRTTPRVTVPARGPPSTLSNASTATSVYSNQTVSTRLGS
Bri  ..V......H...P.S....T...NS..PA...SS..A....P....LH..........
Lor  ..V......H...P.SL...T...NS..SA...SS..A....P....LH..........
Mex  .TS..SMS.G.TSQVVHRTT.NFK.NQAPEFTPSSG.SVRSS..QLTNL.SHSSG-.--.
Tor  .TS..SMS
Nor  SSA..LRS.G.MS---------------.PI.FA-S----KQKQVQS.GI..PNY.-P---
Sou  SSA..LRS.G.MA---------------.PM.VQ-.----KSKTPQS.GF..PAYDMS---
Des  SSA..LRS.G.M.---------------.PTIY..S----KTN.QLS.GF..ANY-MP---
Chi  SSA..LRS.N.MA---------------.PL..Q-.----KQKPL.SEG...PAYD-----

     181
Cam  SAGSGTGVSSFPSTVRTRNWVEDQNR----NLSPFMRGALNTSFVTPPSSRSSNQGTVST
Bri  ..........L..AA...S.....----.....................S.S....
Lor  ..........LS.AA...S.....----.....................S.S.
Mex  RSSGS.V...L..SS...DR.NQ..L-----..E.Y.P.S.RIA.......TA.SS.....
Nor  -----------S.IS..TS...S..SSRFG....YHAE....VWL...G.TA.--S.L.S
Sou  -----------TVSS..SS..QS..SLR--SV...H.Q..Q.VW....G.T..--SS..S
Des  -----------S.VS..SE..SS..
Chi  ------------PVQ..AS..QS..SSR--SW..YH.Q..Q.VW....G.T..--SS...

     241
Cam  VPKEILDSWTGAFNTRRQPLFAHIRKRGESRV
Bri  .....V..........S.............
Lor  ..............................
Mex  ...NV.....SRLTGADSRCLIPL.R....N.
Nor  ..R--------.Y...D.L....NN.R
Sou  T.Y--------.V...D.M....NL.R
Chi  A.R--------.Y...D.L....NL.R
```

Fig. 2. Alignment of amino acids encoded by ORF3. Partial sequences only are shown for Toronto, Hawaii and Desert Shield viruses. Percentage identities are summarized in Table III. The abbreviated virus designations are those used in the Tables. Arrows indicate the sequence shown in Figure 3a.

eleven viruses compared in Tables I and II because of the availability or otherwise of published sequences. Chiba virus is included, whereas OTH-25 and KY-89 are not. The alignment of the deduced amino acid sequences is shown in Figure 2. The level of amino acid identity between viruses was much lower than for ORF1 and ORF2 and yet the viruses were readily divided into the same two genetic groups (Table III). The complete proteins encoded by ORF3 varied in length from 208 to 268 amino acids and contained well-defined deletions and inser-

TABLE III. Percentage Identities in Pairwise Comparisons of Amino Acid Sequences Encoded by ORF3 of SRSVs*

	Cam	Bri	Lor	Mex	Tor	Haw	Nor	Sou	Des
Camberwell 268 aa									
Bristol 268 aa	*90*								
Lordsdale 268 aa	*90*	*98*							
Mexico 252 aa	*43*	*44*	*44*						
Toronto 116 aa (partial)	*54*	*56*	*56*	*97*					
Hawaii 76 aa (partial)	*58*	*61*	*61*	*83*	*87*				
Norwalk 212 aa	29	30	30	23	28	36			
Southampton 211 aa	30	31	31	24	28	34	*68*		
Desert Shield 161 aa (partial)	32	33	34	25	30	33	*63*	*63*	
Chiba 208 aa	31	32	31	24	30	37	*71*	*78*	*58*

*The lengths of sequences compared are indicated by the number of amino acids in column 1. All sequences compared begin at the amino terminus of the deduced amino acid sequence. The percentages indicative of the genotypic groupings are shown in italics.

(a) ORF3

```
                    123                                                 169
                     |                                                   |
Cam  GTRYWAPDARTTTYNAGRFSTSQPSGALPGRTTPRVTVPARGPPSTLSNASTATSVYSNQTVST
Row  ..........................V.....................................
Gis  ..........................V.....................................
UpY  ......................P..P.......N.............................
Fra  ......................P..P.......N..A..........................
Bri  ..........V......H...P.S....T...NS..PA...SS..A....P....LH.......
Lor  ..........V......H...P.SL...T...NS..SA...SS..A....P....LH.......
```

(b) ORF1

```
Cam  GDFKISINEGLPSGVPCTSQWNSIAHWLLTLCALSEVTNLSPDIIQANSLFSFYG
Gis  ..................................................A......
Fra  ..................................................
Bri  ..................................................
Lor  ..................................................
```

Fig. 3. Alignment of partial deduced amino acid sequences for **a)** ORF3 and **b)** ORF1. Amino acid residues 113 to 176 encoded by ORF3 are shown (Bristol and Camberwell virus numbering, see Fig. 2). Residues 253 to 307 encoded by ORF1 are shown (numbering as for Bristol virus partial ORF1, Green et al. [1994]). In b the last two residues listed are the start of the conserved polymerase motif YGDD.

tions (Fig. 2). For example, beginning at residue 30, the first six viruses in Figure 2 had an insertion of eleven amino acids relative to the others. Camberwell, Bristol and Lordsdale viruses had a second insertion of 11 amino acids relative to Mexico, Toronto, and Hawaii viruses beginning at residue 48.

The comparisons between Camberwell, Bristol, and Lordsdale viruses were of particular interest because they are the most closely related set of viruses for which the complete ORF2 and ORF3 sequences are known. The deduced amino acid sequences for ORF3 contained twenty-seven differences between Camberwell and Bristol viruses. Fifteen of these were clustered between residues 123 and 169, i.e., 56% of the differences were contained in a section comprising 18% of the total sequence. In this region of the ORF3 encoded protein, the Camberwell, Bristol, Lordsdale, and Mexico viruses had an insert of approximately 20 amino acids compared with viruses in the other genetic group (Fig. 2). The variation in ORF3 observed among all viruses, and especially in this region for Camberwell and Bristol viruses, suggested that an analysis of ORF3 might provide a sensitive means of distinguishing between virus isolates that otherwise appeared to be closely related or identical by comparison of ORF1 or ORF2 sequences.

Accordingly, primers 2720 and 2721 were used to am-

plify cDNA corresponding to the highly variable region of ORF3 of Bristol and Camberwell viruses from four faecal samples (Fig. 1). These were obtained from outbreaks of vomiting and diarrhoea during the relatively short period from January to November 1994 in the state of Victoria. The outbreaks were restricted to an area extending no further than approximately 60 km from the centre of Melbourne. The results of comparing sequences derived from the ORF3 cDNA and the corresponding sequences of Camberwell, Bristol and Lordsdale viruses are shown in Figure 3a. Camberwell, Rowsley and Gisborne viruses were similar and distinguishable from the closely related pair of Upper Yarra and Frankston viruses. All were distinct from Bristol and Lordsdale viruses.

Also provided in Figure 3b are comparisons for representative viruses of the commonly amplified region of ORF1 defined by primers 51 and 3 [Moe et al., 1994]. The carboxy terminal end of the deduced amino acid sequence for this region contains the first residues of the conserved polymerase motif YGDD. The five viruses in Figure 3b were identical at the amino acid level with the exception of one residue. At the nucleotide level, the Victorian viruses could be readily distinguished from Bristol virus (11 or 10 nucleotide changes, sequences not shown), but not from each other. Thus the results indicated that this selected sequence of ORF3 provided a more sensitive indicator of virus difference than the frequently used region of ORF1.

Clearly there is much to discover regarding the genetic stability and evolution of the human enteric caliciviruses. To obtain evidence regarding the co-circulation of variants and the existence of additional groups or subgroups, more extensive sequencing is required of conserved and variable genomic regions of additional isolates. A useful application of the sequencing of highly variable regions will be the identification of virus variants and the documentation of their spread in disease outbreaks.

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